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Plasma Tissue Factor and Tissue Factor Pathway Inhibitor Levels in Patients With Disseminated Intravascular Coagulation

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We measured the plasma levels of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in patients with disseminated intravascular coagulation (DIC) to examine the relationship between TFPI and vascular endothelial cell injury. Plasma TF (273 ± 90 pg/ml) and TFPI (252 ± 125 ng/ml) levels were significantly increased in patients with DIC compared with non-DIC patients. Plasma TF antigen level was significantly increased in pre-DIC patients (285 ± 85 pg/ml), while the plasma TFPI level (152 ± 54 ng/ml) was not markedly increased in such a state. The plasma TF/TFPI ratio was high in the pre-DIC patients (2.10 ± 0.90), and low in the DIC patients (1.40 ± 0.87) and healthy volunteers (0.84 ± 0.26). There was no significant difference between the DIC patients with a good outcome and those with a poor outcome in terms of plasma TF levels, although the plasma TFPI level in the DIC patients with a good outcome (289 ± 133 ng/ml) was significantly higher than those with a poor outcome (187 ± 75 ng/ml). During the clinical course of DIC, plasma TF antigen was increased first, and an increase of the plasma TFPI level followed the increase in plasma TF level. These findings suggest that plasma TFPI is released from vascular endothelial cells and it may reflect vascular endothelial cell injury. It is conceivable that TF and TFPI may play an important role in the onset of DIC. Am. J. Hematol. 55:169–174, 1997. © 1997 Wiley-Liss, Inc.

Key words: DIC; TFPI; TF; TF/TFPI ratio; outcome

INTRODUCTION

Tissue factor (TF), which serves as the receptor and essential cofactor for factor VII and VIIa [1], is the primary cellular initiator of the coagulation protease cascade. As a potent initiator of coagulation, TF is believed to have a critical function in hemostasis and thrombogenesis [2–4]. Tissue factor pathway inhibitor (TFPI), previously referred to as extrinsic pathway inhibitor [5[or lipoprotein-associated coagulation inhibitor (LACI) [6], is an endogenous anticoagulant protein of the serine protease inhibitor family. TFPI consists of three Kunitz type inhibitory domains [7]; the second Kunitz domain is the FXa inhibitor, while the first domain is responsible for FVIIa/tissue factor (TF) inhibition [8]. Disseminated intravascular coagulation (DIC) [9,10], a condition associated with severe bleeding tendency and organ failure and sometimes exhibiting a very rapid and severe clinical course, appears to be related to vascular endothelial cell injury. Recently, plasma levels of other agents involved in the coagulation cascade, i.e., thrombomodulin (TM), tissue type plasminogen activator (t-PA), plasminogen activator inhibitor-I (PAI-I), and von Willebrand Factor (vWF), all of which are released from vascular endothelial cells, have been reported [11,12]. Plasma TFPI level in patients with DIC was slightly increased or to be within normal range [13], and in those with systemic meningococcal disease it was significantly increased [14]. The change of plasma TFPI level during the clinical course of DIC and the relationship with the prognosis of DIC are still not clear. In this

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TABLE I. Diagnostic Criteria for DIC*

		DIC score (points)
1. PT ratio	1.25–1.66	1
	>1.67	2
2. Fibrinogen (g/l)	1.00–1.50	1
	<1.00	2
3. FDP (μg/ml)	10–20	1
	20–40	2
	>40	3
4. Platelet count (X 10^3/μl)	80–120	1
	50–80	2
	<50	3
5. Symptoms of bleeding	(+)	1
6. Organ failure due to thrombosis	(+)	1

*In leukemic patients, the sum of the scores for 1, 2, 3, and 6 was 4 or higher. In non-leukemic patients, the sum of the scores for 1, 2, 3, 4, 5, and 6 was 7 or higher.

study, we measured the plasma levels of TF and TFPI in patients with DIC to examine the relationship between TFPI and vascular endothelial cell injury.

MATERIALS AND METHODS

Subjects

Our subjects were 79 patients with DIC, 35 non-DIC patients (23 leukemia and 12 non-leukemia), and 10 healthy volunteers. The diagnosis of DIC was based on the criteria established by the Japanese Ministry of Health and Welfare (Table I) [12,15]. In 27 of the DIC patients, hemostatic examination had been carried out within the previous week; these patients we retrospectively termed ''pre-DIC'' [16]. The diseases underlying DIC were leukemia in 57 patients [13 with acute myeloblastic leukemia (AML), 14 with acute promyelocytic leukemia (APL), 6 with acute myelomonocytic leukemia (AMMoL), 5 with chronic myelocytic leukemia, blastic crisis (CML,bc), 12 with acute lymphoblastic leukemia (ALL), and 7 with malignant lymphoma stage IV] and non-leukemic in 22 patients (6 with gastric cancer, 3 with lung cancer, 2 with prostate cancer, 2 with colon cancer, 7 with sepsis, and 2 with gynecological diseases).

DIC patients were treated with gabexate mesilate (FOY), a synthetic proteinase inhibitor [17,18] that inhibits the activity of thrombin, factor Xa, plasmin, and plasma kallikrein. The efficacy of the DIC treatment was assessed after 7 days using the DIC score shown in Table II. We regarded the outcome as good when both the DIC score and the symptoms were improved and the patients survived; the poor outcome was termed when the DIC score increased, the symptoms worsened, or the patients died.

Plasma TF antigen level was measured with an IMUBIND Tissue Factor ELISA kit (ADI, Greenwich, CT). The test specimen was diluted 1:10 in 0.05 M Tris, pH 7.5, with 2% bovine serum albumin and 0.05% Tween 20

TABLE II. Outcome of DIC Patients

	Good (%)	Poor (%)	Total
Leukemic group	40 (70.2)	17 (29.8)	57
Non-leukemic group	11 (50.0)	11 (50.0)	22
Total	51 (64.6)	28 (35.4)	79

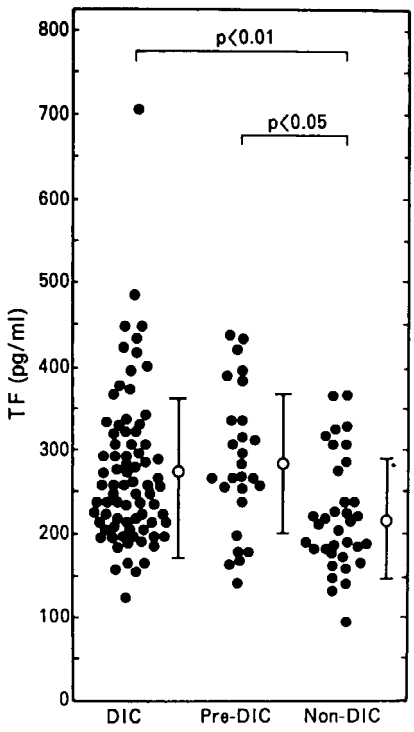

Fig. 1. Plasma TF antigen levels in DIC, pre-DIC, and non-DIC patients.

buffer, to eliminate the matrix effect of undiluted plasma. The monoclonal antibody in this kit was murine immunoglobulin G1 for human brain tissue factor, detecting the TF-apoprotein complex, TF, and TF-FVII complex [19,20]. Plasma TFPI level was measured with an IMUBIND TFPI ELISA kit (ADI) [21]. The murine monoclonal antibody in this kit binds near the Kunitz domain 1, as demonstrated by its binding to a single 43-KDa band of recombinant TFPI (ADI) corresponding to the mobility of TFPI on Western blot analysis.

Values are expressed as means ± standard deviation. The significance of the difference between two groups was assessed by Wilcoxon's nonpaired t-test, and the significance of the differences in the clinical courses of 15 DIC patients (10 leukemic patients and 5 non-leukemic patients) was assessed by Wilcoxon's paired t-test.

RESULTS

Plasma TF (273 ± 90 pg/ml) and TFPI (252 ± 125 ng/ml) levels were significantly increased in patients

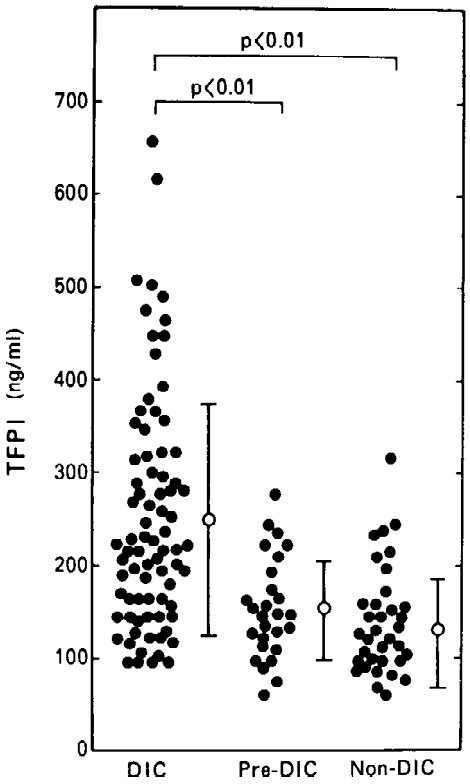

Fig. 2. Plasma TFPI antigen levels in DIC, pre-DIC, and non-DIC patients.

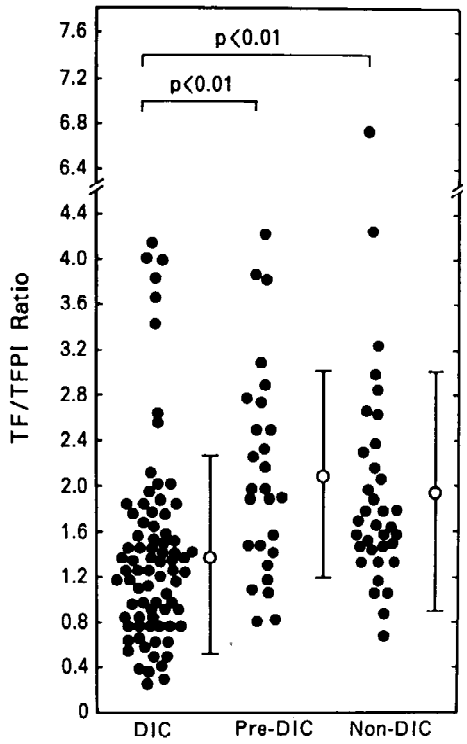

Fig. 3. Plasma TF/TFPI ratio in DIC, pre-DIC, and non-DIC patients.

with DIC compared with the non-DIC patients (TF: 219 ± 68 pg/ml and TFPI: 129 ± 59 ng/ml, $P < 0.01$, respectively) and healthy volunteers (117 ± 19.2 ng/ml and 102 ± 18.5 pg/ml, $P < 0.01$, respectively). In the pre-DIC patients, plasma TF antigen level was significantly increased (285 ± 85 pg/ml) compared with the non-DIC patients, while the plasma TFPI level (152 ± 54 ng/ml) in the pre-DIC patients was not markedly increased (Figs. 1 and 2). The plasma TF/TFPI ratio was high in both the pre-DIC patients (2.10 ± 0.90) and non-DIC patients (1.97 ± 1.08), and was low in the DIC patients (1.40 ± 0.87) ($P < 0.01$, respectively) (Fig. 3). There was no significant difference in plasma TF level between the DIC patients with a good outcome (258 ± 63 pg/ml) and those with a poor outcome (299 ± 120 pg/ml), although the plasma TFPI level in the DIC patients with a good outcome (289 ± 133 ng/ml) was significantly higher than the level in those with a poor outcome (187 ± 75 ng/ml). The TF/TFPI ratio in the DIC patients with a good outcome (1.13 ± 0.66) was significantly lower than that in the patients with a poor outcome (1.83 ± 1.00, $P < 0.01$) (Fig. 4). In the clinical course of the 15 DIC patients, assessed plasma TF antigen was increased in the pre-DIC state (258 ± 65 pg/ml) and at the onset (303 + 77 pg/ml) compared with non-DIC patients; it was slightly reduced on the third day (259 ± 74 pg/ml) and significantly reduced 10 days after the onset (146 ± 37 pg/ml) (Fig. 5). Plasma TFPI level was still not markedly increased in pre-DIC (158 ± 57 ng/ml), especially the patients with poor outcome. Plasma TFPI level was significantly higher in non-DIC patients at the onset of DIC (316 ± 96 ng/ml) and the third day (323 ± 73 ng/ml); however, in patients with poor outcome, TFPI level were not significantly increased (Fig. 6). The plasma TF/TFPI ratio in pre-DIC (1.84 ± 0.81), particularly in patients with poor outcome, was significantly higher than that at the onset of DIC (1.2 ± 0.69, $P < 0.05$), or the third day (0.83 ± 0.25, $P < 0.01$), and on the 10th day (1.25 ± 0.23) (Fig. 7).

DISCUSSION

Since TF is the major initiator of the onset of DIC in acute leukemia [22,23], the presence of high levels of TF in the plasma has been considered to indicate a pathologic state. Indeed, increased plasma TF levels have been reported in DIC patients [20]. Plasma soluble TF is derived from activated macrophages and vascular endothelial cells. In DIC patients, macrophages are activated by cytokines, or endotoxin, and vascular endothelial cells are activated or injured by these chemical mediators [24,25]. However, plasma TF antigen has been detected not only in DIC patients but also in normal volunteers.

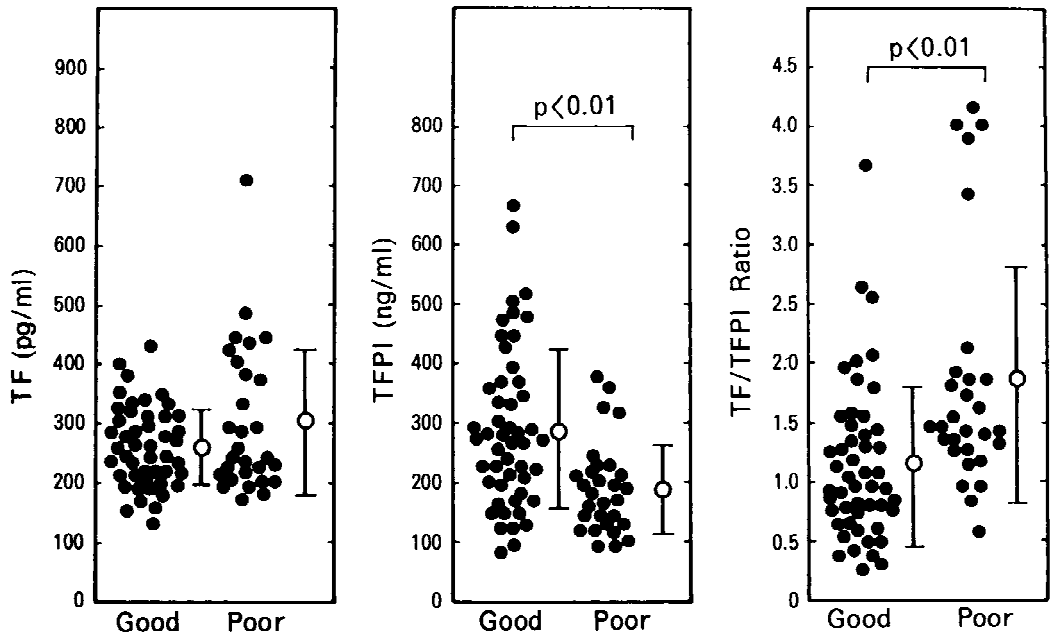

Fig. 4. Plasma TF, TFPI, and TF/TFPI ratio in patients with good and poor outcomes.

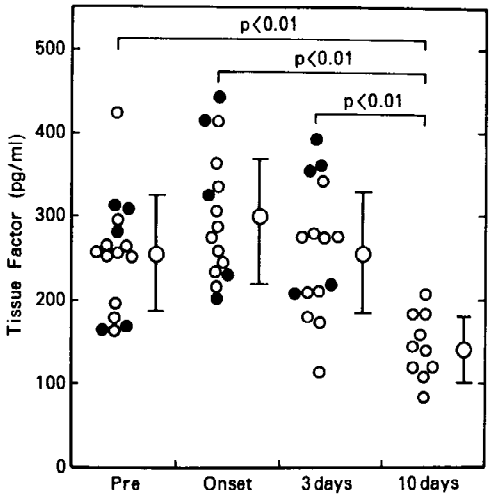

Fig. 5. Plasma TF antigen levels during clinical course of DIC. Open circles: good outcome; closed circles: poor outcome.

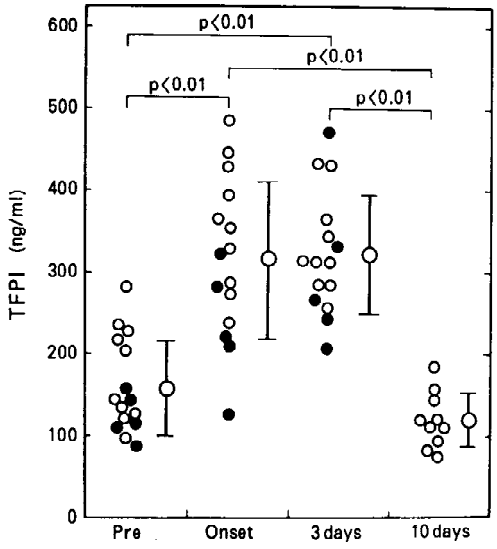

Fig. 6. Plasma TFPI antigen levels during clinical course of DIC. Open circles: good outcome; closed circles: poor outcome.

Plasma TFPI, which inhibits the TF pathway, is considered to play an important role in various hemostatic states. Decreased plasma TFPI levels are reported in patients with TTP [21]. It was reported that plasma TFPI activity in patients with DIC was increased or within normal range and in DIC patients with severe liver disorder it was not decreased [26]. Plasma TFPI is almost exclusively produced in vascular endothelial cells, and it may be present in vascular endothelial cells bound to glycosaminoglycan [27,28]. We found here that plasma TF level was significantly increased in both DIC and pre-DIC patients, while the plasma TFPI level was significantly increased in DIC patients, but was not markedly increased in pre-DIC patients. As the glycosaminoglycan on the endothelial cell decreases in DIC, TFPI can not bind that. It is reported that the carboxy-terminal region mediates TFPI binding to cell surface [29]. In DIC, some proteases may cleave this site of TFPI, following that TFPI lacking carboxy-terminal may be released from vascular endothelial cells.

There was no significant difference in plasma TF levels between the DIC patients with a good outcome and

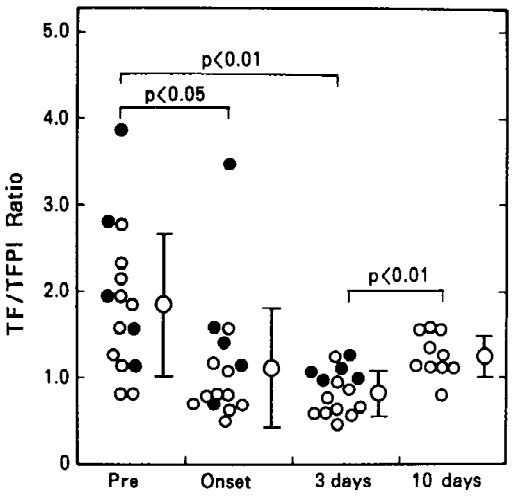

Fig. 7. Plasma TF/TFPI ratio during clinical course of DIC. Open circles: good outcome; closed circles: poor outcome.

those with a poor outcome, although the plasma TFPI level in the DIC patients with a good outcome was significantly higher than the level in those with a poor outcome. Decreased plasma TFPI levels have been reported in patients with TTP [21], in which condition an increase in plasma TM level was reported; severe vascular endothelial damage is also considered to be associated with TTP [11,21]. As plasma TFPI is considered to be derived mainly from vascular endothelial cells, it is possible that the reduction in plasma TFPI level may be caused by severe vascular endothelial cell damage, which phenomenon may explain the poor outcome in DIC and TTP. Plasma TF antigen increased first, and increases in plasma TFPI levels followed the increase in plasma TF levels. In TTP patients after treatment, the TFPI/TF ratio was significantly increased compared with that at the onset [21]. The plasma TF/TFPI ratio was high in the pre-DIC patients in this study, indicating a markedly hypercoagulable state that could progress to DIC. After the onset of DIC, the TF/TFPI ratio became low and the hypercoagulability was improved, indicating that some DIC patients may improve due to the increase of TFPI. There is an approximate 500–1,000-fold molar excess of TFPI molecules related to TF molecules. The ratio of TF/TFPI is inversely correlated to the severity of disease with highest levels in the control subjects. In the DIC group, poor outcome was associated with a lower TFPI response and higher TF/TFPI ratio. These data confirm the role of TF to induce DIC and provide additional evidence that the TF/TFPI ratio may be essential for the regulation of TF induced blood coagulation. It appears that TF and TFPI may play an important role in the onset of DIC. The improvement of DIC may depend, in part, on increases in plasma TFPI, reflecting the condition of vascular endothelial cells.

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